PATENT ABSTRACTS OF JAPAN

(11)Publication number : 2002-315570
(43)Date of publication of application : 29.10.2002

(51)Int.Cl. C12N 1/14
C12N 9/02
// (C12N 1/14
C12R 1:645)

(21)Application number : 2001-125372
(22)Date of filing : 24.04.2001

(71)Applicant : AKIYAMA YUKITO
NAKAMURA TOMOYUKI
(72)Inventor : NAKAMURA TOMOYUKI
AKIYAMA YUKITO

(54) METHOD FOR CULTURING PHELLINUS LINTEUS PRODUCING SOD- LIKE SUBSTANCE AND METHOD FOR PRODUCING SOD-LIKE SUBSTANCE

(57)Abstract:
PROBLEM TO BE SOLVED: To provide a method for culturing Phellinus linteus producing a superoxide dismutase(SOD)-like substance in large amounts and a method for producing the SOD-like substance.
SOLUTION: (1) This method for culturing a mycelium of Phellinus linteus producing the SOD-like substance is characterized by inoculating the mycelium of Phellinus linteus into a liquid medium and culturing the mycelium under conditions of the following (a) and (b): (a) feeding oxygen to the liquid medium during culture; (b) irradiating the liquid medium with light. (2) This SOD-like substance is obtained from the mycelium and the culture solution.

LEGAL STATUS

[Date of request for examination] 28.06.2002
[Date of sending the examiner's decision of rejection]
[Kind of final disposal of application other than the examiner's decision of rejection or application converted registration]
[Date of final disposal for application]
[Patent number]
[Date of registration]
[Number of appeal against examiner's decision of rejection]
[Date of requesting appeal against examiner's decision of rejection]
[Date of extinction of right]

CLAIMS

[Claim(s)]
[Claim 1] Phellinus linteus Phellinus linteus which produces the SOD (superoxide dismutase) Mr. matter characterized by inoculating a mycelium into a liquid medium and cultivating according to following (**) and the conditions of (**) The culture approach of a mycelium.
(b) Supply oxygen to the liquid medium under culture.
(b) Irradiate light at a liquid medium.
[Claim 2] Phellinus linteus Phellinus linteus which produces the SOD Mr. matter characterized by inoculating a mycelium into a liquid medium and cultivating according to following (**) - (Ha) conditions The culture approach of a mycelium.
(b) Supply oxygen to the liquid medium under culture.
(b) Irradiate light at a liquid medium.
(c) Circulate a liquid medium.
[Claim 3] Phellinuslinteus obtained by the culture approach of claim 1 or claim 2 SOD Mr. matter obtained from a mycelium.
[Claim 4] Phellinuslinteus obtained by the culture approach of claim 1 or claim 2 SOD Mr. matter obtained from culture medium.
[Claim 5] Phellinuslinteus obtained by the culture approach of claim 1 or claim 2 The manufacture approach of the SOD Mr. matter characterized by refining a mycelium according to the following process.
A process: The process which extracts a mycelium by ethanol and extracts with ethyl acetate what was obtained at the process B process:A process of removing ethanol [claim 6] Phellinuslinteus obtained by the culture approach of claim 1 or claim 2 The manufacture approach of the SOD Mr. matter characterized by extracting and refining culture medium with ethyl acetate.

[Translation done.]

DETAILED DESCRIPTION

[Detailed Description of the Invention]
[0001]
[Industrial Application] This invention is Phellinus linteus which produces the superoxide dismutase (SOD) Mr. matter so much. It is related with the culture approach and the manufacture approach of the SOD Mr. matter.
[0002]
[Description of the Prior Art] SOD (superoxide dismutase) is an enzyme which eliminates the super oxide (O2 and −) which is one of active oxygen and the free radicals (reduction), and is changed into a hydrogen peroxide (H2 O2). Conventionally, it looks forward to development of drugs and food which the various illnesses in which the rise and fall of the active oxygen produced in the living body participate are known, and singlet oxygen (1O2), super oxide, a hydrogen peroxide, a hydroxy radical (HO−), etc. take in inside of the body, and eliminate active oxygen.
[0003]
[Problem(s) to be Solved] When the artificer etc. was studying the approach of acquiring the mycelium of Phellinus linteus (Phellinus Linteus) in large quantities in a liquid medium and he cultivated, irradiating light at a culture medium, in a mycelium and culture medium, he knew that the SOD Mr. matter would be produced so much, and completed this invention.
[0004]
[Means for Solving the Problem] The invention in this application is constituted by the claim of following (1) − (6).

Claim 1 :P hellinus linteus Phellinus linteus which produces the SOD (superoxide dismutase) Mr. matter characterized by inoculating a mycelium into a liquid medium and cultivating according to following (**) and the conditions of (**) The culture approach of a mycelium.
(b) Supply oxygen to the liquid medium under culture.
(b) Irradiate light at a liquid medium.
Claim 2 :P hellinus linteus Phellinus linteus which produces the SOD Mr. matter characterized by inoculating a mycelium into a liquid medium and cultivating according to following (**) − (Ha) conditions The culture approach of a mycelium.
(b) Supply oxygen to the liquid medium under culture.
(b) Irradiate light at a liquid medium.
(c) Circulate a liquid medium.
Claim 3: Phellinus linteus obtained by the culture approach of claim 1 or claim 2 SOD Mr. matter obtained from a mycelium.
Claim 4: Phellinus linteus obtained by the culture approach of claim 1 or claim 2 SOD Mr. matter obtained from culture medium.
Claim 5: Phellinus linteus obtained by the culture approach of claim 1 or claim 2 The manufacture approach of the SOD Mr. matter characterized by refining a mycelium according to the following process.
A process: Phellinus linteus obtained by the culture approach of of process claim 6:claim 1 or claim 2 which extracts a mycelium by ethanol and extracts with ethyl acetate what was obtained at the process B process:A process of removing ethanol The manufacture approach of the SOD Mr. matter characterized by extracting and refining culture medium with ethyl acetate.
[0005]
[Embodiment of the Invention] (b) Phellinus linteus (Phellinus Linteus) used for the invention in this application used what was saved as PL−08 share after being Suki, Nishi−Morokata−gun, Miyazaki−ken−mura, extracting the fruit body and mycelium−izing at an incorporated company eye BI eye application mushroom lab in October, 1998. this strain −− a fruit body −− research institute, the Forestry Agency, the Ministry of Agriculture, Forestry, and Fisheries Department of a forest living thing section forest microorganism having the cystidium of a yellowish brown color peculiar to ** Phellinus Linteus fruit body by judgment of Dr. Yukihisa Abe of a decay disease laboratory, and the gestalt of ** basidiospore −− since −− what was identified Phellinus Linteus was

used. The preculture of sample offering strain inoculated into the Potato Dextrose Agar culture medium in a Petri dish with a bore of 90mm (Difco shrine make) the mycelium which had carried out cold storage at 5 degrees C, and carried out surface culture for 15 days under 25-degree-C dark. This culture mycelium was cut off with the cork borer with a bore of 5mm (desiccation mycelium weight equivalent to 0.35mg), and the trial was presented.
[0006] (1) The effectiveness of the light to mycelium growth of Phellinus linteus (Phellinus Linteus) was investigated.
The culture condition is as follows. Phellinus linteus (Phellinus Linteus) was inoculated into the 500ml Erlenmeyer flask which poured the 300ml of the following culture media distributively, and sterilized, aeration of the sterile air which let 0.22-micrometer filter pass was carried out at a rate of 0.5 l/min to it, and the cell mass of Phellinus Linteus which grew up was measured.
(b) Medium composition Glucose: 4%, yeast extractives:0.3%, peptone:0.3% KH2 PO4 : 0.05%, Na2 HPO4 : 0.05% (pH5.5)
(b) A culture condition (a) -- culture temperature: -- 25 degrees C (b) -- culture scale: -- a 500ml Erlenmeyer flask and 300ml of culture media (c) -- quantity-of-airflow: -- 0.5 l/min (d) -- culture days: -- for 40 days (e) Exposure conditions : Light source : The LED light source made from EYELA, red LED (660nm) illuminance: Eight following partitions of 0-4000LUX 0, 100, 500, 1000, 1500, 2000 3000 4000 (LUX)
Irradiation time: 8 hours / DAY, [0007] The result is shown in drawing 1 (Phellinus linteus radiation effects of the light in mycelium (Phellinus Linteus) growth). According to drawing 1 , when exposure reinforcement exceeds 1000 (LUX), it turns out that fungus body yield decreases. In addition, when the light source used for this invention is not limited to the above-mentioned light source and the usual visible ray was irradiated, it was enough, but when blue LED (470nm) was irradiated, it was in the inclination bronzed while growth of a mycelium is overdue (aging), and the mycelium growth of the far-infrared color LED (735nm) suited to the late inclination.
[0008] (2) Phellinus Linteus was cultivated on condition that the medium composition of said (**), and following (**), and the desiccation mycelium of Phellinus linteus (Phellinus Linteus) was obtained (10.2 g/L).
This obtained mycelium is displayed as "PL-L" below.
(b) Medium composition : it is the same as said (b).
(b) Culture condition (a) culture temperature: 25 degrees C (b) culture scale: 1000L (refer to drawing 2)
(c) Quantity of airflow : 35 L/min (d) culture days: 20 days (e) exposure conditions: Light source: The LED light source made from EYELA, red LED (660nm)
Illuminance: 1000LUX Irradiation time: 8 hours / DAY Illuminance: Phellinus linteus (Phellinus Linteus) was cultivated and the desiccation mycelium was obtained without irradiating light on the other hand using the same medium composition as the above, and culture condition (a) - (d) 1000 LUX (8.3 g/L). This obtained mycelium is displayed as "PL-nonL" below. Moreover, "PL-nonL-20day" expresses the mycelium cultivated on the 20th, without irradiating light.
[0009] (c) The centrifugal separator separated the mycelium cultivated the above condition, ethanol was added to this, the mycelium was crushed by the mixer, and it put for 24 hours. After collecting ethanol fractionation with the centrifugal separator after that, removing ethanol and ethyl acetate's extracting residue, ethyl acetate was removed and it considered as the sample for antioxidation active substance measurement. In addition, the sample for antioxidation active substance measurement was prepared also about what processed the culture medium (medium) before the Phellinus Linteus inoculation with ethanol and ethyl acetate like the mycelium. two samples ("PL-nonL-20day", "PL-L-20day") of said mycelium, and the ethanol solution of "medium" -- electron spin resonance (ESR) -- it asked for the rate of super oxide (O2 and -) elimination (SOD Mr. operation) by law. electron spin resonance (ESR) -- measurement of the super oxide (O2 and -) by law the approach (Kazuo Ouchi and edit: -- living thing pharmaceutical chemistry experiment lecture inflammation and allergy [ ] -- p.218-p.223 (Hirokawa bookstore) --) using a spin-trapping agent and DMPO (5 and 5-dimethyl-pyrroline-N-oxide) It carried out by BulL.Chem.Soc.Jpn., 63,187-191 (1990), BIOCHEMISTRY and MOLECULAR BIOLOGY INTERNATIONAL Vol.42, No.1, and P.35-44 (1997).
[0010] The result is shown in drawing 3 . According to drawing 3 , the mycelium (PL-nonL-20day) cultivated without the mycelium (PL-L-20day) of Phellinus linteus (Phellinus Linteus) which irradiated light and cultivated it irradiating light shows that the value of the rate of super oxide (O2 and -) elimination is large.
[0011] (3) Phellinus linteus of a paragraph 0008 The concentration test of the SOD Mr. matter was performed about the mycelium and culture filtrate which were obtained by culture (Phellinus Linteus) (what irradiated light).
(b) About the mycelium, 4 fractionation of "PL-F1", "PL-F2", "PL-F3", and "PL-F4" was obtained with the procedure of drawing 4 .
(b) Moreover, about the culture filtrate except a mycelium, 3 fractionation of "PL-F5", "PL-F6", and "PL-F7" was obtained with the procedure of drawing 5 .
[0012] a part for 7-minute formation of these "PL-F1" - "PL-F7" -- a paragraph 0009 -- the same -- electron spin resonance (ESR) -- it asked for the rate of super oxide (O2 and -) elimination (SOD Mr. operation) by law. The result is shown in drawing 6 . As for the value of the rate of super oxide (O2 and -) elimination, according to

drawing 6 , it turns out that the value for 2-minute formation of "PL-F2" and "PL-F6" is large as compared with other components.

[0013] in addition, electron spin resonance (ESR) -- the actual measurement chart of the super oxide (O2 and -) by law is shown in drawing 7 - drawing 18 . The measurement result from which drawing 7 - drawing 10 serve as a basis of the rate (%) calculation of super oxide elimination shown in drawing 3 , drawing 11 - drawing 18 are the measurement charts used as the calculation basis of the rate of super oxide elimination (%) shown in drawing 6 .

[0014]

[Effect of the Invention] As mentioned above, according to the culture approach of Phellinus linteus (Phellinus Linteus) which produces the SOD Mr. matter of the invention in this application, and the manufacture approach of the SOD Mr. matter, while being able to make the mycelium culture of Phellinus linteus (Phellinus Linteus), and its culture medium produce the SOD Mr. matter so much, it has the effectiveness referred to as being able to condense the produced SOD Mr. matter efficiently.

[Translation done.]

DESCRIPTION OF DRAWINGS

[Brief Description of the Drawings]
[Drawing 1] Phellinus linteus It is drawing showing the optical radiation effects in mycelium (Phellinus Linteus) growth.
[Drawing 2] It is this schematic drawing of the culture apparatus of Phellinus linteus (Phellinus Linteus).
[Drawing 3] It is drawing showing the value of the rate of super oxide (O2 and -) elimination.
[Drawing 4] It is this schematic drawing showing the procedure which condenses the SOD Mr. matter from a mycelium.
[Drawing 5] It is this schematic drawing showing the procedure which condenses the SOD Mr. matter from culture medium.
[Drawing 6] It is drawing showing the value of the rate of super oxide (O2 and -) elimination.
[Drawing 7] sample additive-free super oxide -- electron spin resonance (ESR) -- it is drawing (control) measured by law.
[Drawing 8] the super oxide elimination ability of "medium" -- electron spin resonance (ESR) -- law -- constant -- it is a Fig. the bottom.
[Drawing 9] the super oxide elimination ability of "PL-nonL-20day" -- electron spin resonance (ESR) -- it is drawing measured by law.
[Drawing 10] the super oxide elimination ability of "PL-L-20day" -- electron spin resonance (ESR) -- it is drawing measured by law.
[Drawing 11] sample additive-free super oxide -- electron spin resonance (ESR) -- it is drawing (control) measured by law.
[Drawing 12] the super oxide elimination ability of "PL-F1" -- electron spin resonance (ESR) -- it is drawing measured by law.
[Drawing 13] the super oxide elimination ability of "PL-F2" -- electron spin resonance (ESR) -- it is drawing measured by law.
[Drawing 14] the super oxide elimination ability of "PL-F3" -- electron spin resonance (ESR) -- it is drawing measured by law.
[Drawing 15] the super oxide elimination ability of "PL-F4" -- electron spin resonance (ESR) -- it is drawing measured by law.
[Drawing 16] the super oxide elimination ability of "PL-F5" -- electron spin resonance (ESR) -- it is drawing measured by law.
[Drawing 17] the super oxide elimination ability of "PL-F6" -- electron spin resonance (ESR) -- it is drawing measured by law.
[Drawing 18] the super oxide elimination ability of "PL-F7" -- electron spin resonance (ESR) -- it is drawing measured by law.

(19)日本国特許庁（ＪＰ）　(12)**公開特許公報**（Ａ）　(11)特許出願公開番号
特開2002－315570
（P2002－315570A）
(43)公開日　平成14年10月29日(2002.10.29)

(51)Int.Cl.⁷	識別記号	FI		テーマコード(参考)
Ｃ１２Ｎ　1/14		Ｃ１２Ｎ　1/14	Ｂ	４Ｂ０５０
9/02		9/02		４Ｂ０６５
//（Ｃ１２Ｎ　1/14		Ｃ１２Ｒ　1:645		
Ｃ１２Ｒ　1:645）				

審査請求　有　　請求項の数６　ＯＬ　（全 19 頁）

(21)出願番号　特願2001－125372(P2001－125372)

(22)出願日　平成13年４月24日(2001.4.24)

(71)出願人　500003176
秋山　幸仁
山梨県韮崎市円野町上円井1891
(71)出願人　500003165
中村　友幸
山梨県東八代郡八代町岡592
(72)発明者　中村　友幸
山梨県東八代郡八代町岡592
(72)発明者　秋山　幸仁
山梨県韮崎市円野町上円井1891
(74)代理人　100083817
弁理士　今野　耕哉　（外１名）

最終頁に続く

(54)【発明の名称】　ＳＯＤ様物質を生産するＰｈｅｌｌｉｎｕｓｌｉｎｔｅｕｓの培養方法及びＳＯＤ様物質の製造方法

(57)【要約】
【目的】　この発明は、スーパーオキサイドジスムターゼ（ＳＯＤ）様物質を多量に生産するPhellinus linteus （メシマコブ）の培養方法及びＳＯＤ様物質の製造方法に関するものである。
【構成】（１）Phellinus linteus の菌糸体を液体培地に接種して、下記の（イ）及び（ロ）の条件により培養することを特徴とするＳＯＤ（スーパーオキシドジスムターゼ）様物質を生産するPhellinus linteus の菌糸体の培養方法。
（イ）培養中の液体培地に酸素を供給すること。
（ロ）液体培地に光を照射すること。
（２）前記菌糸体及び培養液から得られるＳＯＤ様物質

1

【特許請求の範囲】

【請求項１】　Phellinus linteus の菌糸体を液体培地に接種して、下記の（イ）及び（ロ）の条件により培養することを特徴とするＳＯＤ（スーパーオキサイドジスムターゼ）様物質を生産するPhellinus linteus の菌糸体の培養方法。

（イ）培養中の液体培地に酸素を供給すること。

（ロ）液体培地に光を照射すること。

【請求項２】　Phellinus linteus の菌糸体を液体培地に接種して、下記の（イ）～（ハ）の条件により培養することを特徴とするＳＯＤ様物質を生産するPhellinus linteus の菌糸体の培養方法。

（イ）培養中の液体培地に酸素を供給すること。

（ロ）液体培地に光を照射すること。

（ハ）液体培地を対流させること。

【請求項３】　請求項１又は請求項２の培養方法により得られるPhellinuslinteus の菌糸体から得られるＳＯＤ様物質。

【請求項４】　請求項１又は請求項２の培養方法により得られるPhellinuslinteus の培養液から得られるＳＯＤ様物質。

【請求項５】　請求項１又は請求項２の培養方法により得られるPhellinuslinteus の菌糸体を下記の工程により精製することを特徴とするＳＯＤ様物質の製造方法。

Ａ工程：菌糸体をエタノールで抽出し、エタノールを除去する工程

Ｂ工程：Ａ工程で得られたものを酢酸エチルで抽出する工程

【請求項６】　請求項１又は請求項２の培養方法により得られるPhellinuslinteus の培養液を酢酸エチルで抽出して精製することを特徴とするＳＯＤ様物質の製造方法。

【発明の詳細な説明】

【０００１】

【産業上の利用分野】この発明は、スーパーオキサイドジスムターゼ（ＳＯＤ）様物質を多量に生産するPhellinus linteus の培養方法及びＳＯＤ様物質の製造方法に関するものである。

【０００２】

【従来の技術】ＳＯＤ（スーパーオキサイドジスムターゼ）は、活性酸素・フリーラジカルのひとつであるスーパーオキサイド（O2･- ）を消去（還元）して、過酸化水素（H2 O2 ）に変える酵素である。従来、一重項酸素（ 1O2 ）、スーパーオキサイド、過酸化水素、ヒドロキシラジカル（HO･ ）等、生体内に生ずる活性酸素の消長が関与する多種多様の疾病が知られており、体内に摂取して活性酸素を消去する医薬品や食品の開発が待望されている。

【０００３】

【解決しようとする課題】発明者等は、Phellinus linteus （メシマコブ）の菌糸体を、液体培地中で大量に取得する方法を研究している際、培地に光を照射しながら培養すると、菌糸体、及び培養液中にＳＯＤ様物質が多量に生産されることを知り本発明を完成した。

【０００４】

【課題を解決するための手段】本願発明は下記の（１）～（６）の請求項により構成されている。

請求項１：Phellinus linteus の菌糸体を液体培地に接種して、下記の（イ）及び（ロ）の条件により培養することを特徴とするＳＯＤ（スーパーオキサイドジスムターゼ）様物質を生産するPhellinus linteus の菌糸体の培養方法。

（イ）培養中の液体培地に酸素を供給すること。

（ロ）液体培地に光を照射すること。

請求項２：Phellinus linteus の菌糸体を液体培地に接種して、下記の（イ）～（ハ）の条件により培養することを特徴とするＳＯＤ様物質を生産するPhellinus linteus の菌糸体の培養方法。

（イ）培養中の液体培地に酸素を供給すること。

（ロ）液体培地に光を照射すること。

（ハ）液体培地を対流させること。

請求項３：請求項１又は請求項２の培養方法により得られるPhellinus linteus の菌糸体から得られるＳＯＤ様物質。

請求項４：請求項１又は請求項２の培養方法により得られるPhellinus linteus の培養液から得られるＳＯＤ様物質。

請求項５：請求項１又は請求項２の培養方法により得られるPhellinus linteus の菌糸体を下記の工程により精製することを特徴とするＳＯＤ様物質の製造方法。

Ａ工程：菌糸体をエタノールで抽出し、エタノールを除去する工程

Ｂ工程：Ａ工程で得られたものを酢酸エチルで抽出する工程

請求項６：請求項１又は請求項２の培養方法により得られるPhellinus linteus の培養液を酢酸エチルで抽出して精製することを特徴とするＳＯＤ様物質の製造方法。

【０００５】

【発明の実施の形態】（イ）本願発明に用いたPhellinus linteus （メシマコブ）は、１９９８年１０月に宮崎県西諸県郡須木村で、子実体を採取し、株式会社アイ・ビー・アイ応用きのこ研究所で菌糸体化した上でＰＬ－０８株として保存していたものを使用した。この菌株は、子実体を農林水産省林野庁総合研究所　森林生物部森林微生物科　腐朽病害研究室の阿部恭久博士の鑑定により、①メシマコブ子実体に特有の黄褐色の剛毛体を持つこと、及び②担子胞子の形態、からメシマコブと同定されたものを用いた。供試菌株の前培養は、５℃で低温保存してあった菌糸体を、内径９０mmのペトリ皿内のPotato Dextrose Agar培地（Difco 社製）へ接種して、

２５℃暗黒下で１５日間表面培養した。この培養菌糸体を内径５mmのコルクボーラーで切り取り（乾燥菌糸体重量　０．３５mgに相当）、試験に供した。

【０００６】（１）Phellinus linteus （メシマコブ）の菌糸体成長に対する光の効果を調べた。

（イ）培地組成

グルコース：４%，イーストエキス：０．３%，ペプトン：０．３%，

KH2 PO4 ：０．０５%，Na2 HPO4 ：０．０５%（pH５．５）

（ロ）培養条件

（ａ）培養温度：２５℃

（ｂ）培養規模：５００mlの三角フラスコ，培地３００ml

（ｃ）通気量：０．５l／ｍｉｎ

（ｄ）培養日数：４０日

（ｅ）照射条件：

光源：ＥＹＥＬＡ社製ＬＥＤ光源、赤色ＬＥＤ（６６０nm）

照度：下記の０～４０００ＬＵＸの８区分

０，１００，５００，１０００，１５００，２０００，

３０００，４０００（ＬＵＸ）

照射時間：８時間／ＤＡＹ，

【０００７】その結果を図１（Phellinus linteus （メシマコブ）菌糸体成長における光の照射効果）に示す。図１によれば、照射強度が１０００（ＬＵＸ）を越えると、菌体収量が減少することがわかる。なお、本発明に用いる光源は上記の光源に限定されるものではなく、通常の可視光線を照射すれば十分であるが、青色ＬＥＤ（４７０nm）を照射すると、菌糸体の成長が遅れると共に褐色化（老化）する傾向にあり、遠赤外色ＬＥＤ（７３５nm）は菌糸体成長が遅い傾向にあった。

【０００８】（２）メシマコブを、前記（イ）の培地組成と下記の（ロ）の条件で培養し、Phellinus linteus （メシマコブ）の乾燥菌糸体を得た（１０．２ｇ／Ｌ）。

得られたこの菌糸体を、以下「ＰＬ－Ｌ」と表示する。

（イ）培地組成：前記（イ）と同じ。

（ロ）培養条件

（ａ）培養温度：２５℃

（ｂ）培養規模：１０００Ｌ（図２参照）

（ｃ）通気量：３５Ｌ／ｍｉｎ

（ｄ）培養日数：２０日

（ｅ）照射条件：

光源：ＥＹＥＬＡ社製ＬＥＤ光源、赤色ＬＥＤ（６６０nm）

照度：１０００ＬＵＸ

照射時間：８時間／ＤＡＹ

照度：１０００ＬＵＸ

一方、前記と同じ培地組成及び培養条件（ａ）～（ｄ）を用い、光を照射しないで、Phellinus linteus （メシマコブ）を培養し、乾燥菌糸体を得た（８．３ｇ／Ｌ）。得られたこの菌糸体を、以下「ＰＬ－ｎｏｎＬ」と表示する。又、例えば「ＰＬ－ｎｏｎＬ－２０ｄａｙ」は、光を照射せずに２０日培養した菌糸体を表す。

【０００９】（ハ）以上の条件で培養した菌糸体を、遠心分離機により分離し、これにエタノールを加えて、ミキサーにより菌糸体を破砕し、２４時間静置した。その後遠心分離機によりエタノール分画を集め、エタノールを除去し、残渣を酢酸エチルで抽出した後、酢酸エチルを除去して、抗酸化活性物質測定用サンプルとした。なお、メシマコブ接種前の培養基（ｍｅｄｉｕｍ）を菌糸体と同様にエタノールと酢酸エチルで処理したものについても抗酸化活性物質測定用サンプルを調製した。前記菌糸体の２試料（「ＰＬ－ｎｏｎＬ－２０ｄａｙ」，「ＰＬ－Ｌ－２０ｄａｙ」）及び，「ｍｅｄｉｕｍ」のエタノール溶液について、電子スピン共鳴（ＥＳＲ）法により、スーパーオキサイド（O2･- ）消去率（ＳＯＤ様作用）を求めた。電子スピン共鳴（ＥＳＲ）法によるスーパーオキサイド（O2･- ）の測定は、スピントラッピング剤，ＤＭＰＯ（5,5-dimethyl-pyrroline-N-oxide）を用いる方法（大内和雄・編集：生物薬化学実験講座　炎症とアレルギー　ｐ．２１８－ｐ．２２３（広川書店），BulL.Chem.Soc.Jpn.,63,187-191(1990)，BIOCHEMISTRY and MOLECULAR BIOLOGY INTERNATIONAL Vol.4

2,No.1,P.35-44,(1997)）で行なった。

【００１０】その結果を図３に示す。図３によれば、光を照射して培養したPhellinus linteus（メシマコブ）の菌糸体（ＰＬ－Ｌ－２０ｄａｙ）は、光を照射しないで培養した菌糸体（ＰＬ－ｎｏｎＬ－２０ｄａｙ）よりも、スーパーオキサイド（O2･-）消去率の値が大きくなっていることがわかる。

【００１１】（３）段落０００８のPhellinus linteus（メシマコブ）培養（光りを照射したもの）により得られた菌糸体と培養濾液について、ＳＯＤ様物質の濃縮試験を行った。

（イ）菌糸体については、図４の手順により、「ＰＬ－Ｆ１」，「ＰＬ－Ｆ２」，「ＰＬ－Ｆ３」，「ＰＬ－Ｆ４」の４分画を得た。

（ロ）又、菌糸体を除いた培養濾液については、図５の手順により、「ＰＬ－Ｆ５」，「ＰＬ－Ｆ６」，「ＰＬ－Ｆ７」の３分画を得た。

【００１２】これらの「ＰＬ－Ｆ１」～「ＰＬ－Ｆ７」の７分画成分について、段落０００９と同様に、電子スピン共鳴（ＥＳＲ）法により、スーパーオキサイド（O2･-）消去率（ＳＯＤ様作用）を求めた。その結果を図６に示す。図６によれば、スーパーオキサイド（O2･-）消去率の値は、「ＰＬ－Ｆ２」及び「ＰＬ－Ｆ６」の２分画成分の値が、他の成分に比較して大きくなっていることがわかる。

【００１３】なお、電子スピン共鳴（ＥＳＲ）法によるスーパーオキサイド（O2･-）の実際の測定チャートを図７～図１８に示す。図７～図１０は、図３に示したスーパーオキサイド消去率（％）算出の根拠となる測定結果、図１１～図１８は、図６に示したスーパーオキサイド消去率（％）の算出根拠となる測定チャートである。

【００１４】

【発明の効果】以上のように、本願発明のＳＯＤ様物質を生産するPhellinus linteus（メシマコブ）の培養方法及びＳＯＤ様物質の製造方法によれば、Phellinus linteus（メシマコブ）の菌糸体培養物及びその培養液にＳＯＤ様物質を多量に生産させることができると共に、生産されたＳＯＤ様物質を効率よく濃縮することができると言う効果を有する。

【図面の簡単な説明】

【図１】Phellinus linteus（メシマコブ）菌糸体成長における光照射効果を示す図である。

【図２】Phellinus linteus（メシマコブ）の培養装置の該略図である。

【図３】スーパーオキサイド（O2･-）消去率の値を示す図である。

【図４】菌糸体からＳＯＤ様物質を濃縮する手順を示す該略図である。

【図５】培養液からＳＯＤ様物質を濃縮する手順を示す該略図である。

【図６】スーパーオキサイド（O2･-）消去率の値を示す図である。

【図７】試料無添加のスーパーオキサイドを電子スピン共鳴（ＥＳＲ）法で測定した図（コントロール）である。

【図８】「ｍｅｄｉｕｍ」のスーパーオキサイド消去能を電子スピン共鳴（ＥＳＲ）法で定した図である。

【図９】「ＰＬ－ｎｏｎＬ－２０ｄａｙ」のスーパーオキサイド消去能を電子スピン共鳴（ＥＳＲ）法で測定した図である。

【図１０】「ＰＬ－Ｌ－２０ｄａｙ」のスーパーオキサイド消去能を電子スピン共鳴（ＥＳＲ）法で測定した図である。

【図１１】試料無添加のスーパーオキサイドを電子スピン共鳴（ＥＳＲ）法で測定した図（コントロール）である。

【図１２】「ＰＬ－Ｆ１」のスーパーオキサイド消去能を電子スピン共鳴（ＥＳＲ）法で測定した図である。

【図１３】「ＰＬ－Ｆ２」のスーパーオキサイド消去能を電子スピン共鳴（ＥＳＲ）法で測定した図である。

【図１４】「ＰＬ－Ｆ３」のスーパーオキサイド消去能を電子スピン共鳴（ＥＳＲ）法で測定した図である。

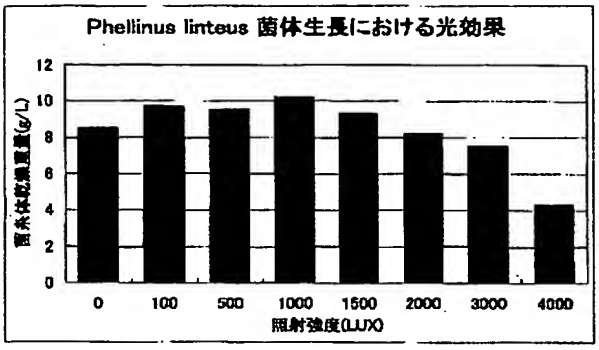
【図１５】「ＰＬ－Ｆ４」のスーパーオキサイド消去能を電子スピン共鳴（ＥＳＲ）法で測定した図である。

【図１６】「ＰＬ－Ｆ５」のスーパーオキサイド消去能を電子スピン共鳴（ＥＳＲ）法で測定した図である。

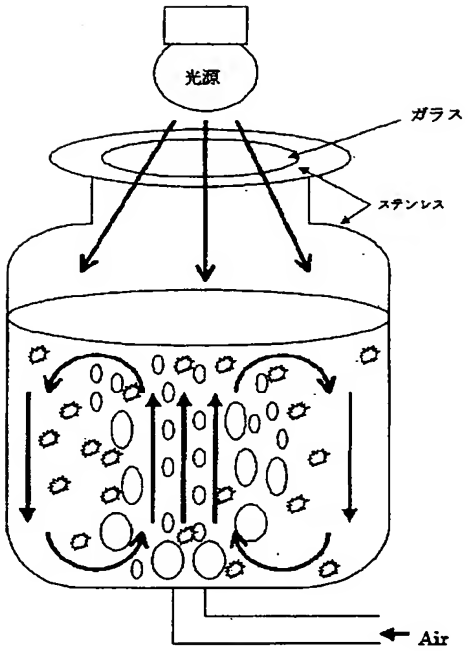
【図１７】「ＰＬ－Ｆ６」のスーパーオキサイド消去能を電子スピン共鳴（ＥＳＲ）法で測定した図である。

【図１８】「ＰＬ－Ｆ７」のスーパーオキサイド消去能を電子スピン共鳴（ＥＳＲ）法で測定した図である。

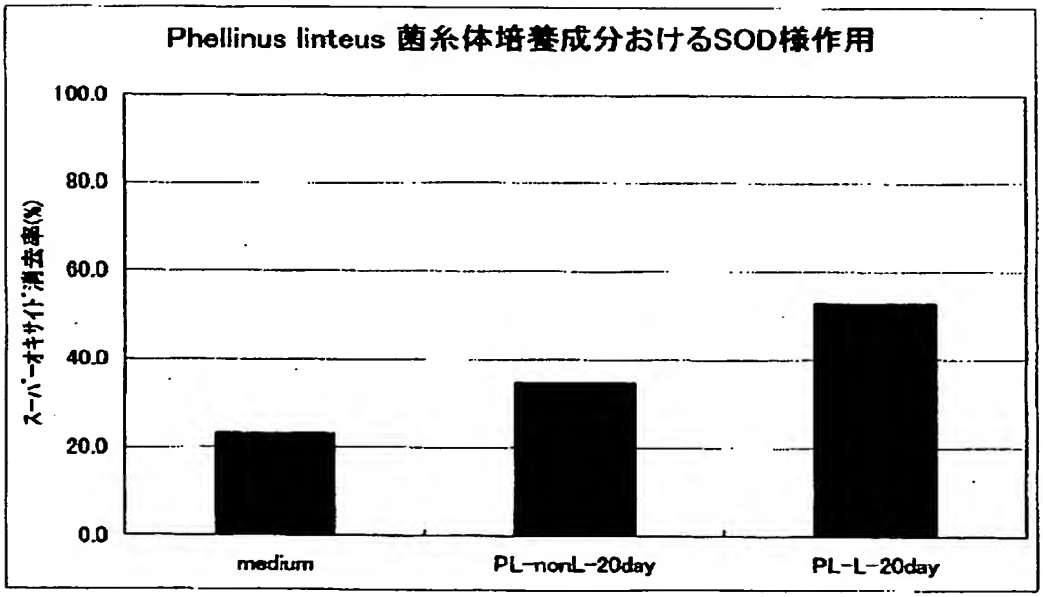
【図１】


Phellinus linteus 菌体生長における光効果
菌糸体乾燥重量(g/L)
0
2
4
6
8
10
12
0
100
500
1000
1500
2000
3000
4000
照射強度(LUX)

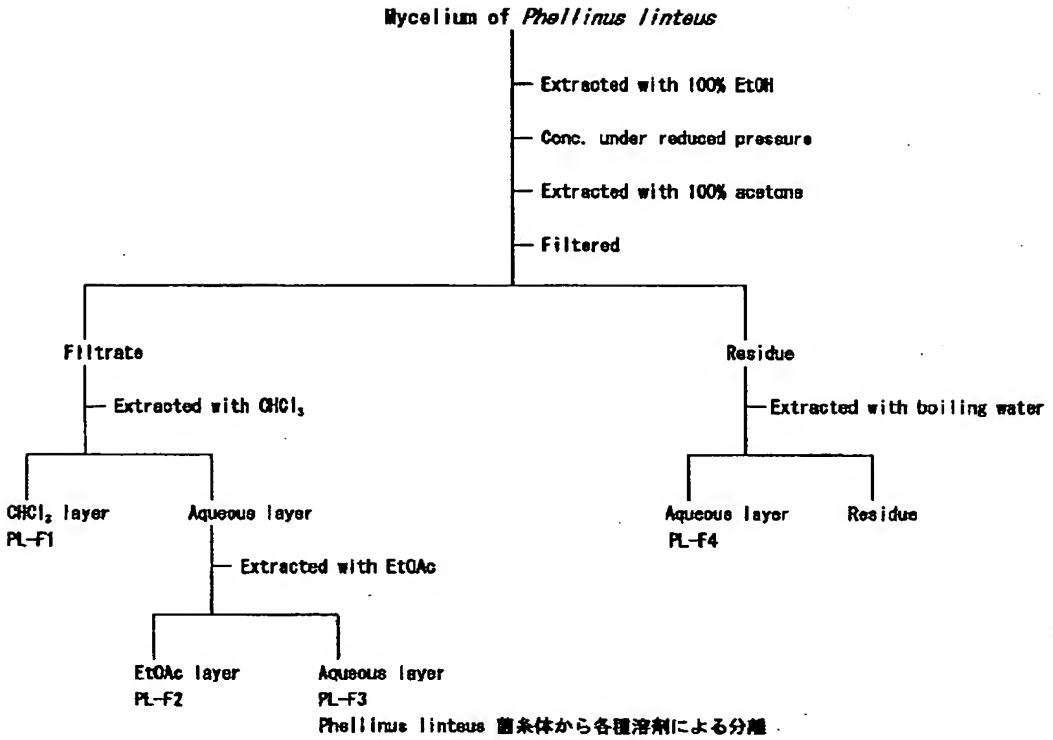
【図２】


光源
ガラス
ステンレス
Air

【図３】

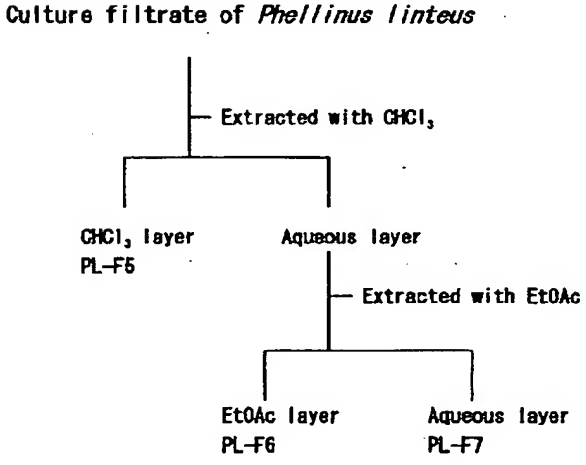

Phellinus linteus 菌糸体培養成分におけるSOD様作用
スーパーオキサイド消去率(%)
0.0
20.0
40.0
60.0
80.0
100.0
medium
PL-nonL-20day
PL-L-20day

【図４】



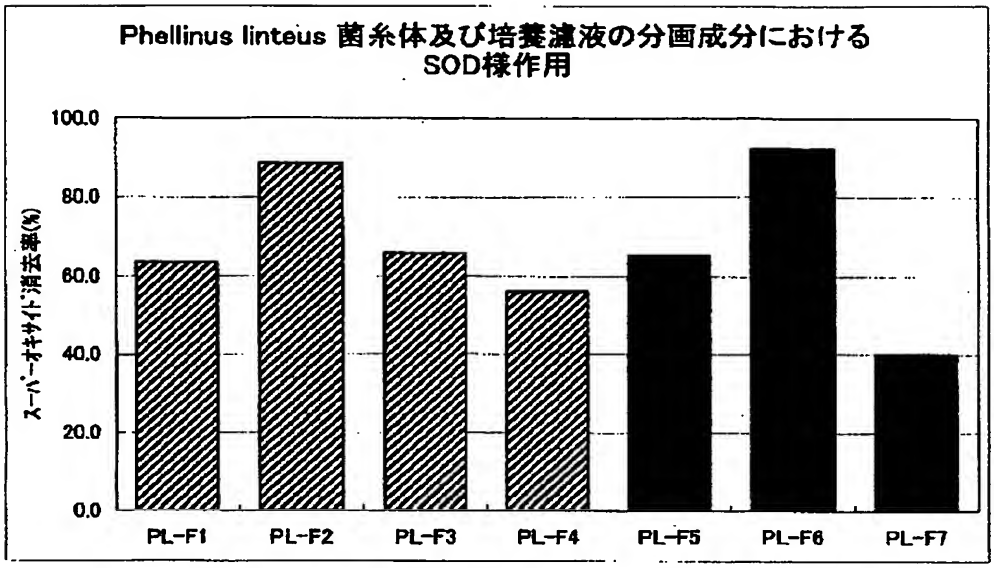
Phellinus linteus 菌糸体から各種溶剤による分離

【図５】

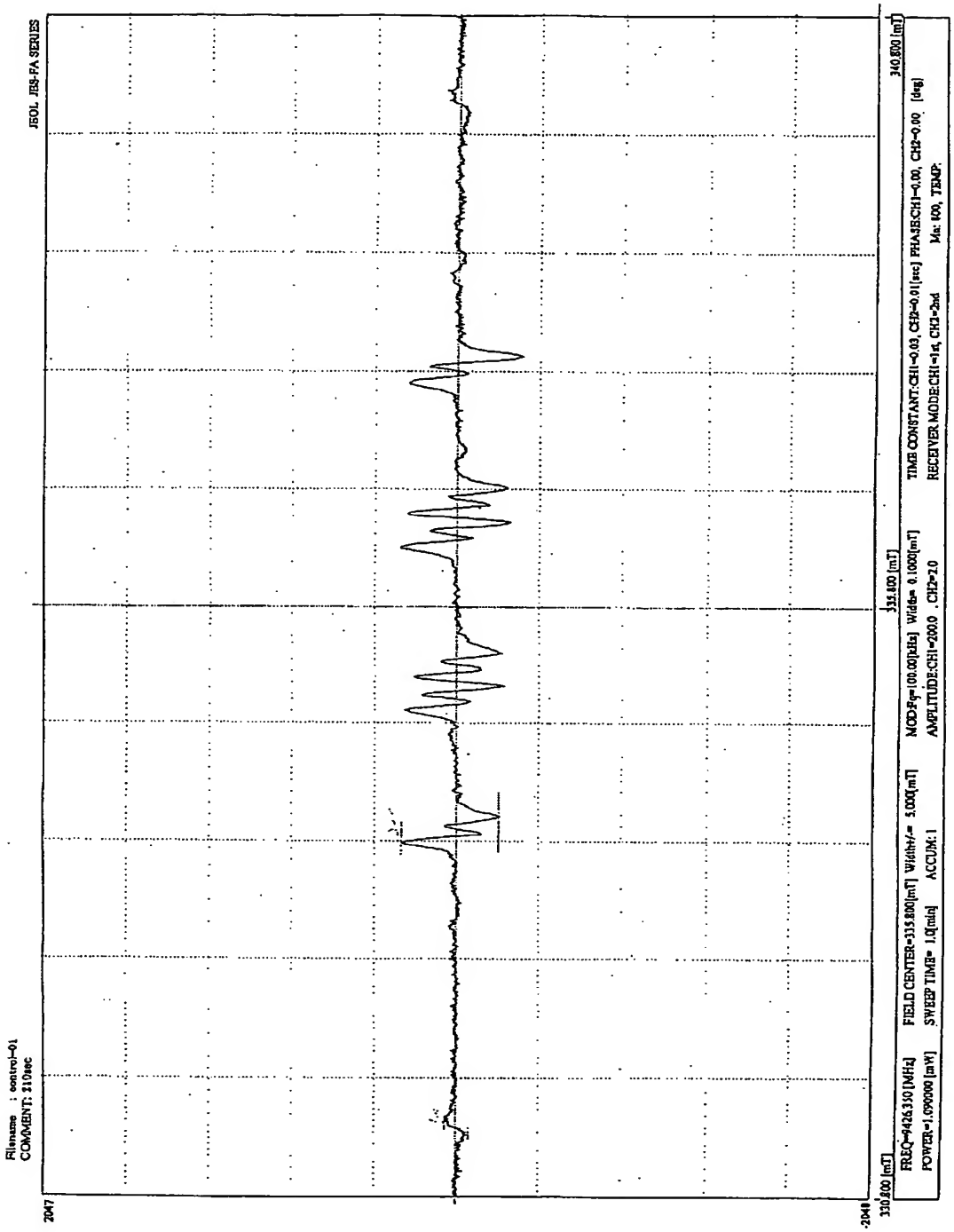


Phellinus linteus 培養濾液から各種溶剤による分離

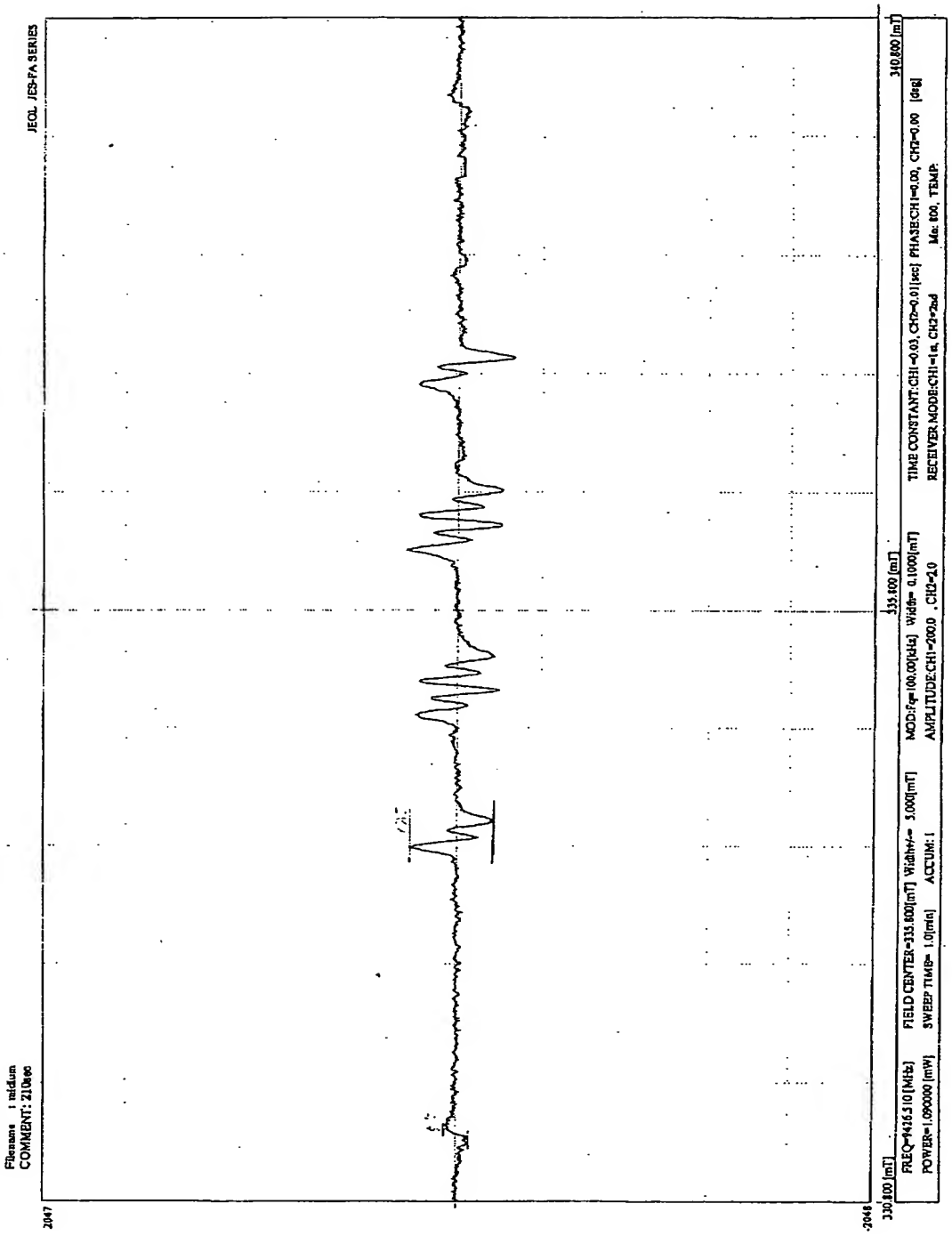
【図６】


Phellinus linteus 菌糸体及び培養濾液の分画成分における
SOD様作用
スーパーオキサイド消去率(%)
100.0
80.0
60.0
40.0
20.0
0.0
PL-F1
PL-F2
PL-F3
PL-F4
PL-F5
PL-F6
PL-F7

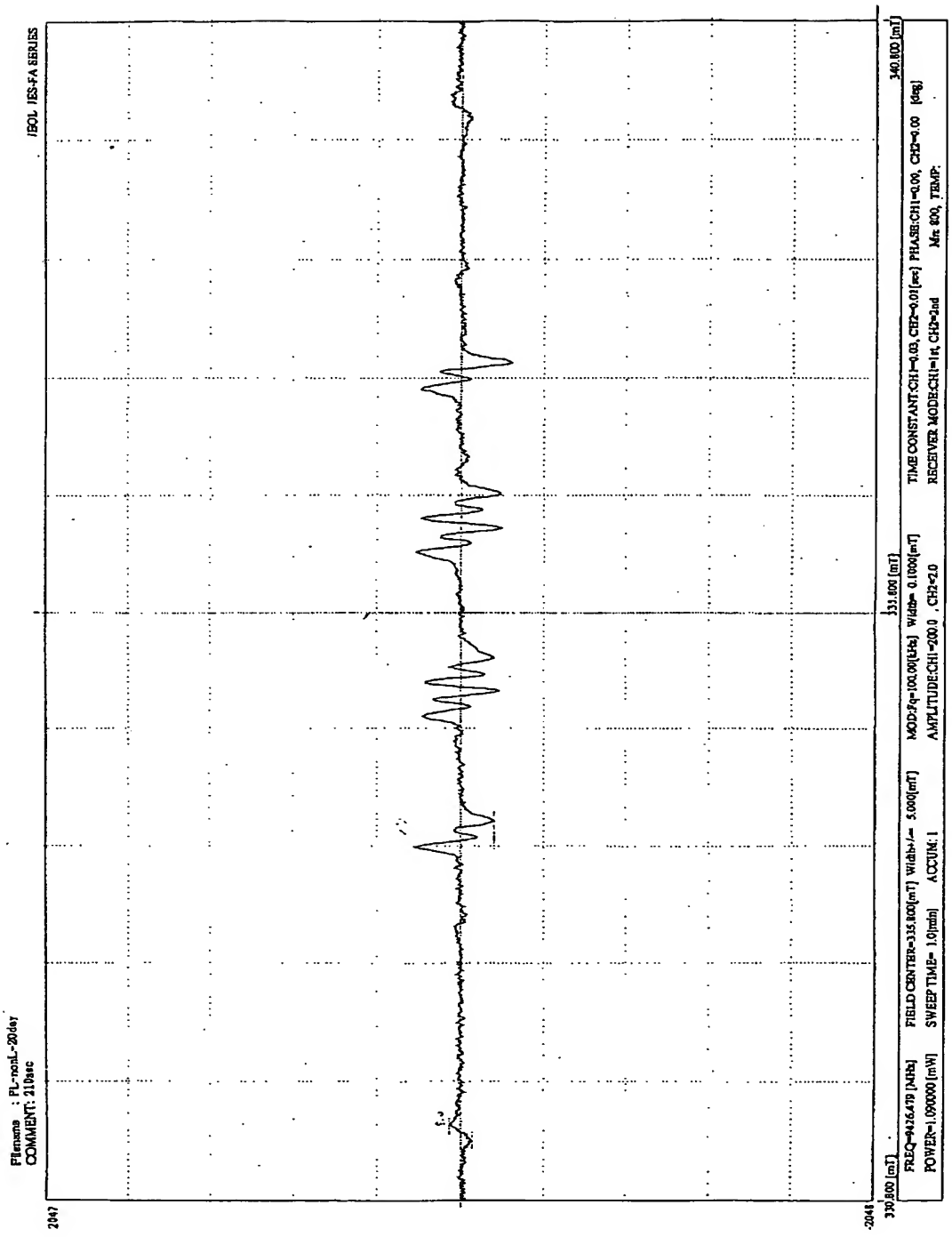
【図７】


JEOL JES-FA SERIES
Filename : control-01
COMMENT: 210sec
2047
-2048
335.800 [mT]
330.800 [mT]
340.800 [mT]
FREQ=9426.330 [MHz]
FIELD CENTER=335.800[mT] Width+/-= 5.000[mT]
MOD-Fq=100.00[kHz] Width= 0.1000[mT]
TIME CONSTANT:CH1=0.03, CH2=0.01[sec] PHASE:CH1=0.00, CH2=0.00 [deg]
POWER=1.000000 [mW]
SWEEP TIME= 1.0[min]
ACCUM: 1
AMPLITUDE:CH1=200.0 , CH2=2.0
RECEIVER MODE:CH1=1st, CH2=2nd
Mx: 400, TEMP:

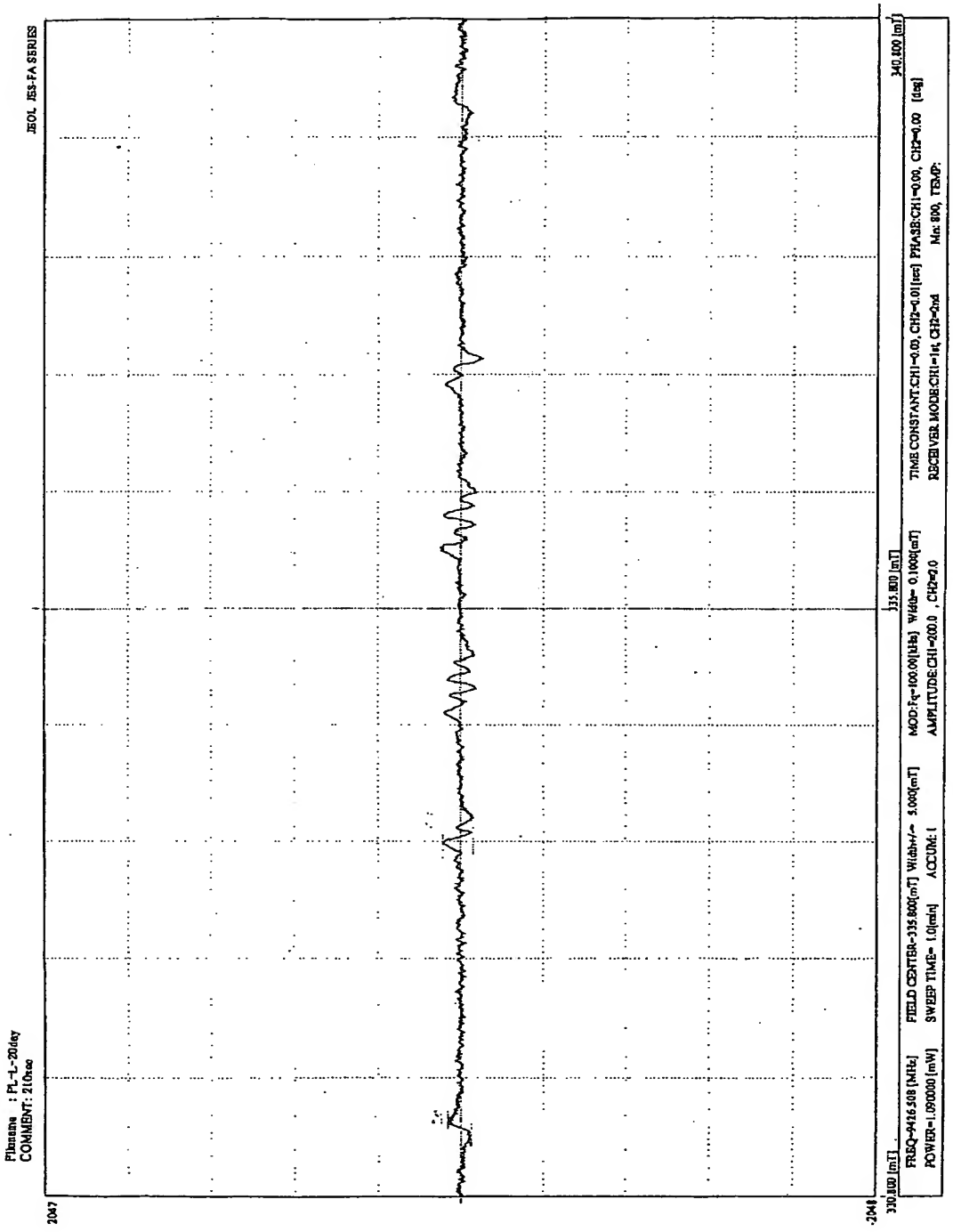
【図８】


JEOL JES-FA SERIES
Filename : midium
COMMENT: 21Dec
2047
-2048
330.800 [mT]
340.800 [mT]
335.800 [mT]
FREQ=9416.510 [MHz]
FIELD CENTER=335.800[mT] Width/+- 5.000[mT]
MOD:Fm=100.00[kHz] Width= 0.1000[mT]
TIME CONSTANT:CH1=0.03, CH2=0.01[sec] PHASE:CH1=0.00, CH2=0.00 [deg]
POWER=1.000000 [mW]
SWEEP TIME= 1.0[min]
ACCUM: 1
AMPLITUDE:CH1=200.0 , CH2=2.0
RECEIVER MODE:CH1=1st, CH2=2nd
Mic 800, TEMP:

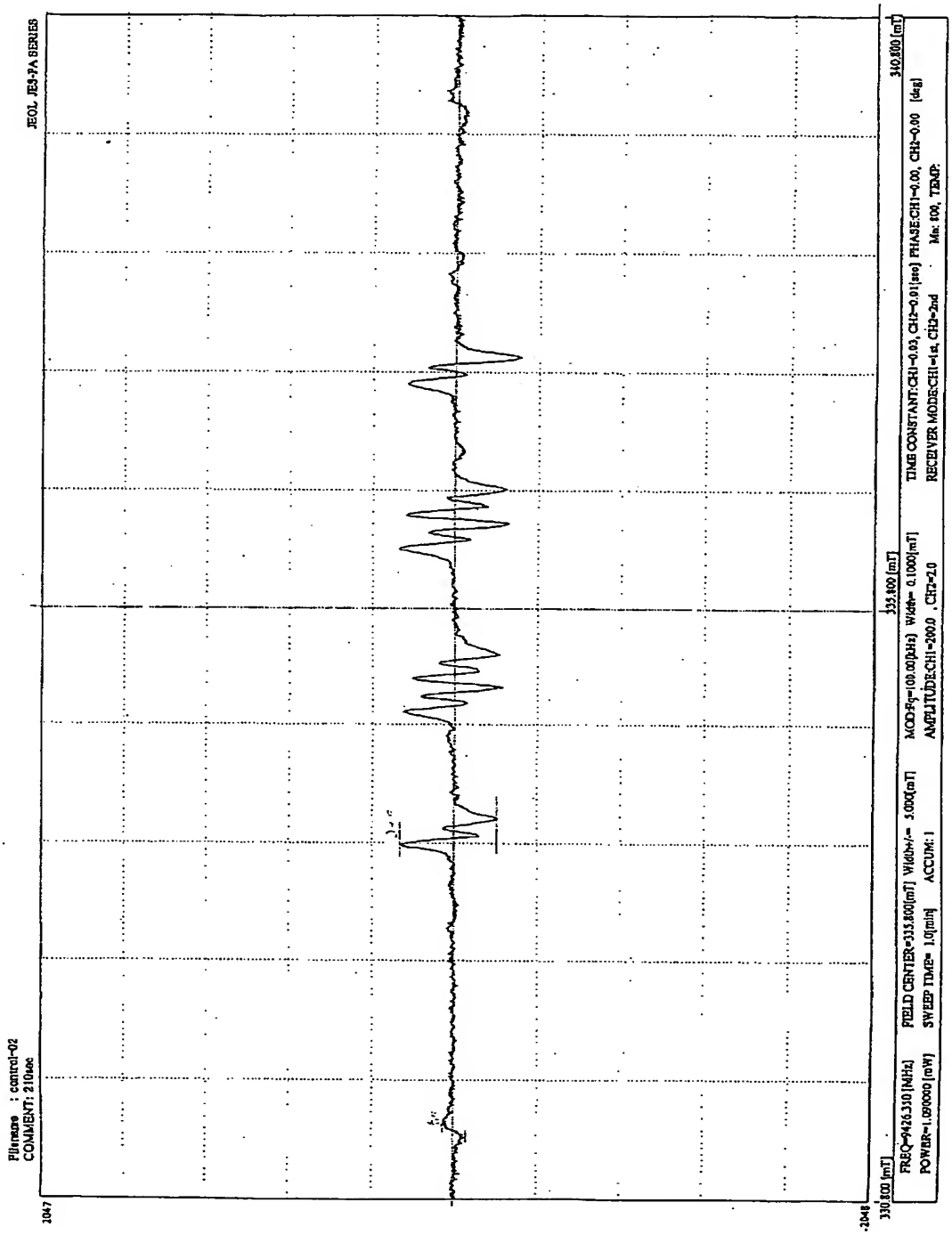
【図９】


JEOL JES-FA SERIES
Filename : PL-nonL-20day
COMMENT: 21Dasc
2047
-2048
330.800 [mT]
335.800 [mT]
340.800 [mT]
FREQ=9426.479 [MHz]
FIELD CENTER=335.800[mT] Width+/-= 5.000[mT]
MOD-Fq=100.00[kHz] Width= 0.1000[mT]
TIME CONSTANT:CH1=0.03, CH2=0.01[sec] PHASE:CH1=0.00, CH2=0.00 [deg]
POWER=1.000000 [mW]
SWEEP TIME= 1.0[min]
ACCUM.: 1
AMPLITUDE:CH1=200.0 , CH2=2.0
RECEIVER MODE:CH1=1st, CH2=2nd
Mn 800, TEMP:

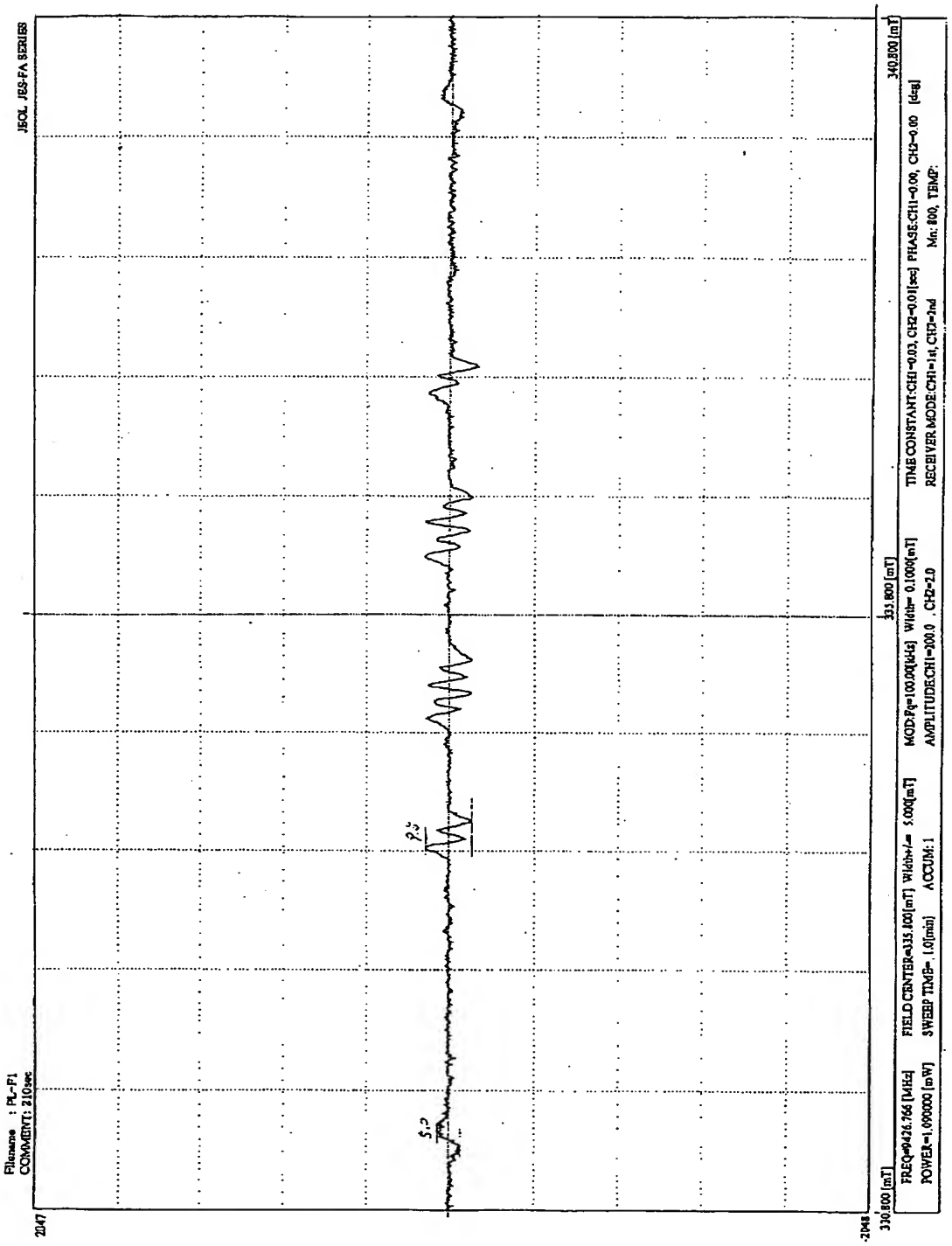
【図１０】


JEOL JES-FA SERIES
Filename : PL-L-20day
COMMENT: 210sec
2047
-2048
335.800 [mT]
330.800 [mT]
340.800 [mT]
FREQ=9416.508 [MHz]
POWER=1.000000 [mW]
FIELD CENTER=335.800[mT] Width+/- 5.000[mT]
SWEEP TIME= 1.0[min] ACCUM: 1
MOD:Fq=100.00[kHz] Width= 0.1000[mT]
AMPLITUDE:CH1=200.0 ,CH2=0.0
TIME CONSTANT:CH1=0.03, CH2=0.01[sec] PHASE:CH1=0.00, CH2=0.00 [deg]
RECEIVER MODE:CH1=1st, CH2=2nd Mx: 800, TEMP:

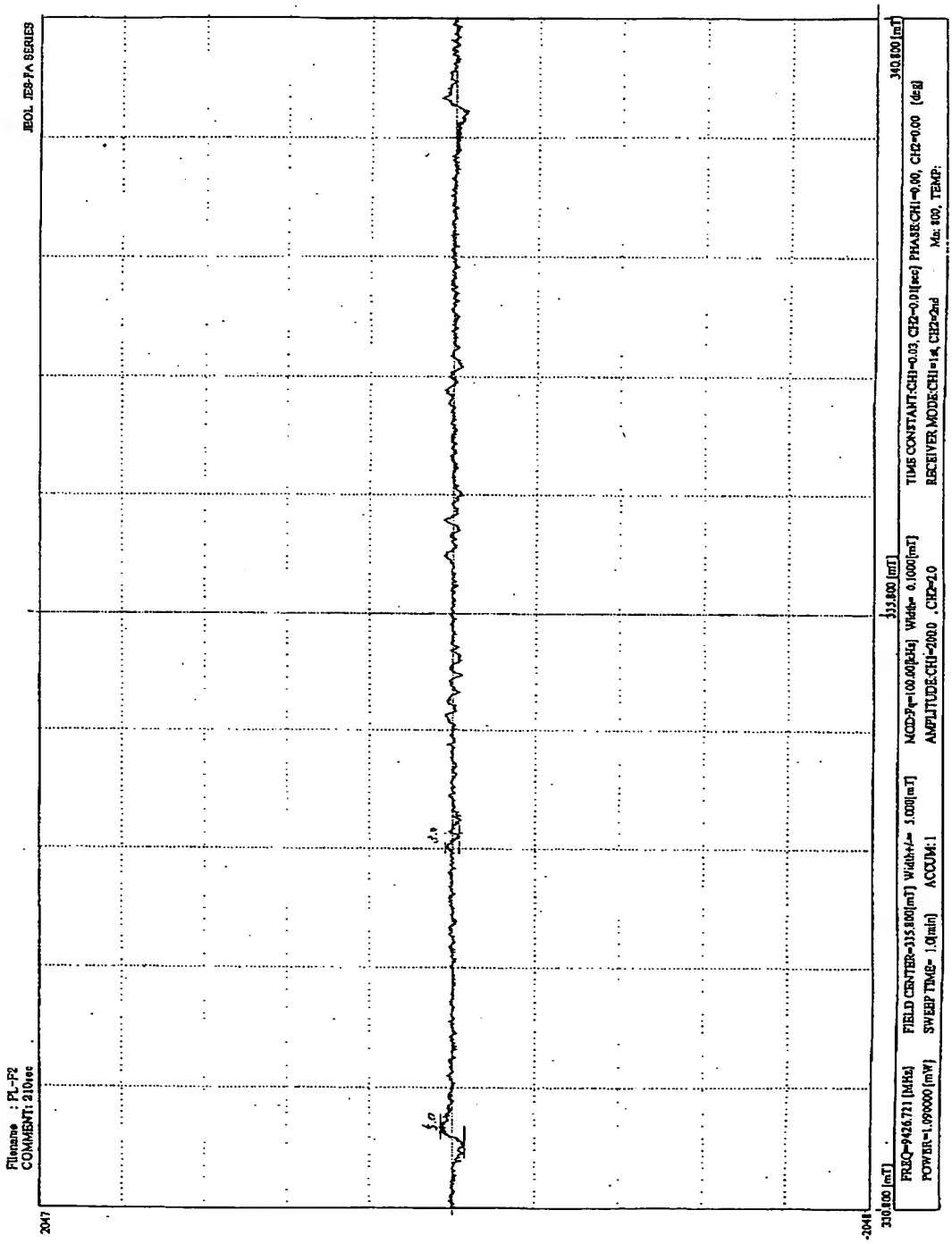
【図１１】


JEOL JES-FA SERIES
Filename : control-02
COMMENT: 210sec
1047
-2048
330.800 [mT]
335.800 [mT]
340.800 [mT]
FREQ=9426.330 [MHz]
FIELD CENTER=335.800[mT] Width/div= 5.000[mT]
MOD:Fm=100.00[kHz] Width= 0.1000[mT]
TIME CONSTANT:CH1=0.03, CH2=0.01[sec] PHASE:CH1=0.00, CH2=0.00 [deg]
POWER=1.000000 [mW]
SWEEP TIME= 1.0[min]
ACCUM: 1
AMPLITUDE:CH1=200.0 , CH2=2.0
RECEIVER MODE:CH1=1st, CH2=2nd
Max 400, TEMP:

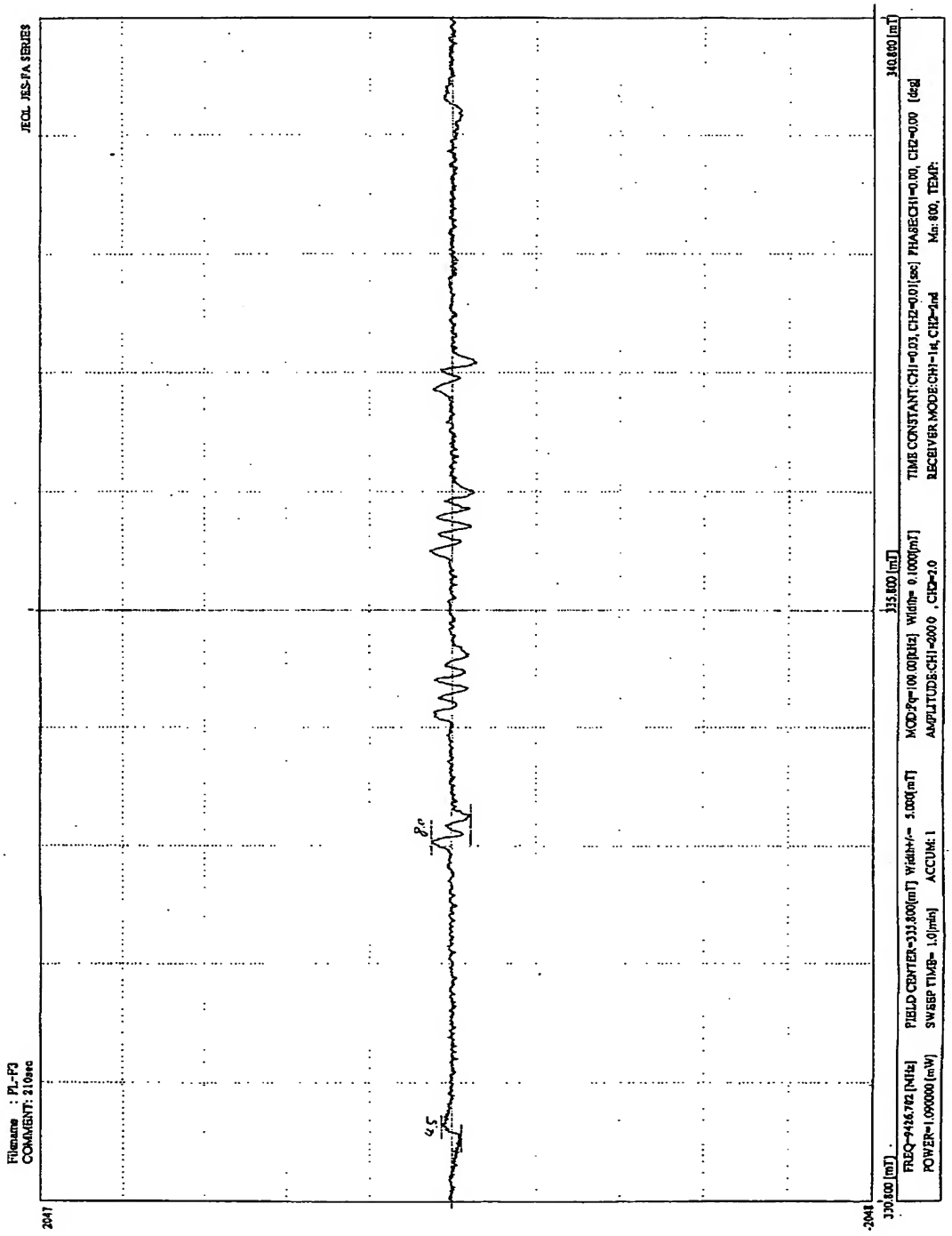
【図１２】


JEOL JES-FA SERIES
Filename : PL-F1
COMMENT1: 210sec
2047
-2048
335.800 [mT]
330.800 [mT]
340.800 [mT]
FREQ=9426.766 [MHz]
POWER=1.000000 [mW]
FIELD CENTER=335.400[mT] Width=5.000[mT]
SWEEP TIME= 1.0[min] ACCUM: 1
MOD:Fq=100.00[kHz] Width= 0.1000[mT]
AMPLITUDE:CH1=200.0 , CH2=2.0
TIME CONSTANT:CH1=0.03, CH2=0.01[sec] PHASE:CH1=0.00, CH2=0.00 [deg]
RECEIVER MODE:CH1=1st, CH2=2nd Mn: 800, TEMP:

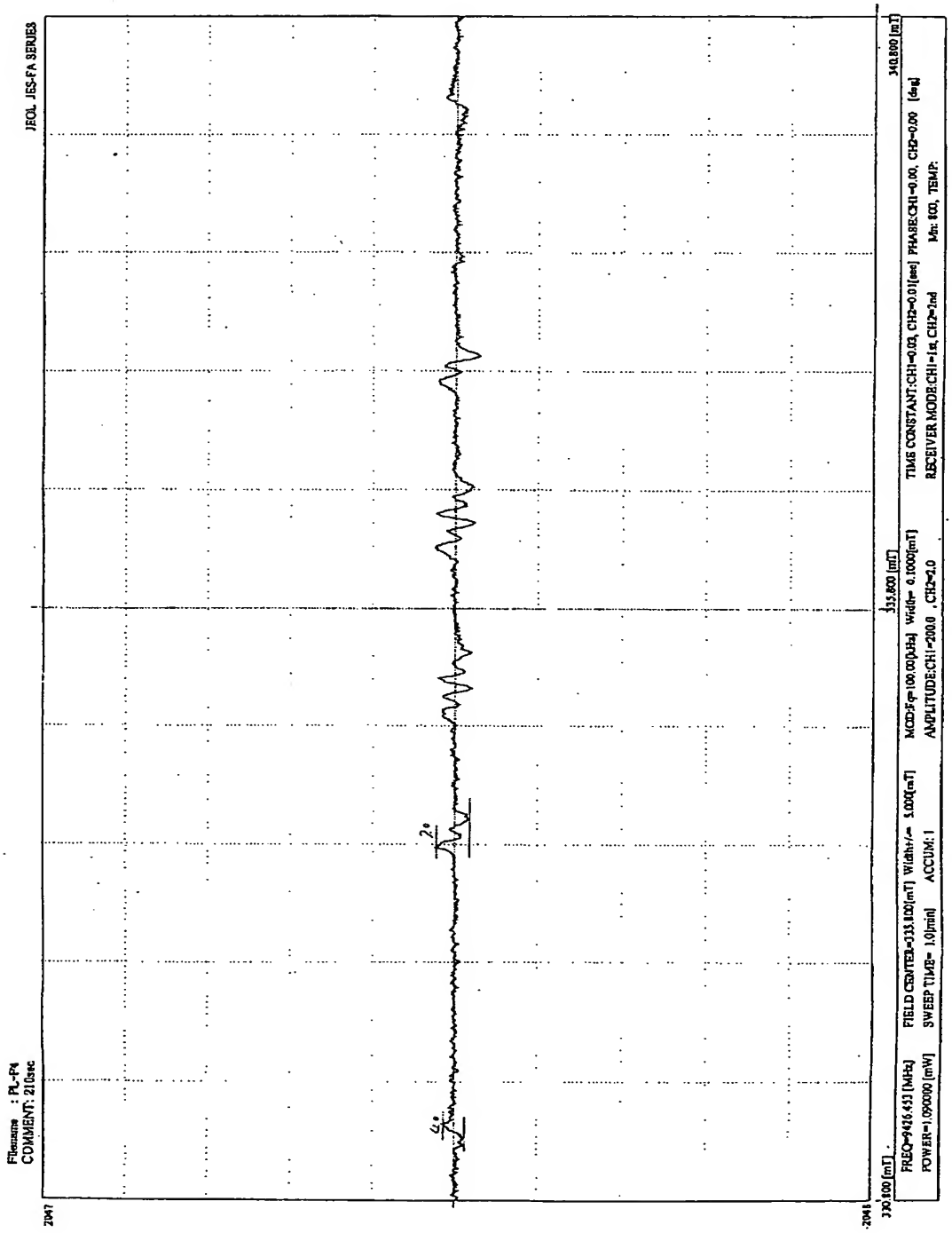
【図１３】


JEOL JES-FA SERIES
Filname : PL-P2
COMMENT: 210sec
2047
-2048
330.800 [mT]
340.800 [mT]
335.800 [mT]
FREQ=9426.721 [MHz]
FIELD CENTER=335.800[mT] Width+/-= 5.000[mT]
MOD.F=100.00[kHz] Width= 0.1000[mT]
TIME CONSTANT:CH1=0.03, CH2=0.01[sec] PHASE:CH1=0.00, CH2=0.00 [deg]
POWER=1.990000 [mW]
SWEEP TIME= 1.0[min]
ACCUM: 1
AMPLITUDE:CH1=200.0 ,CH2=1.0
RECEIVER MODE:CH1=1st, CH2=2nd
Ma: 800, TEMP:

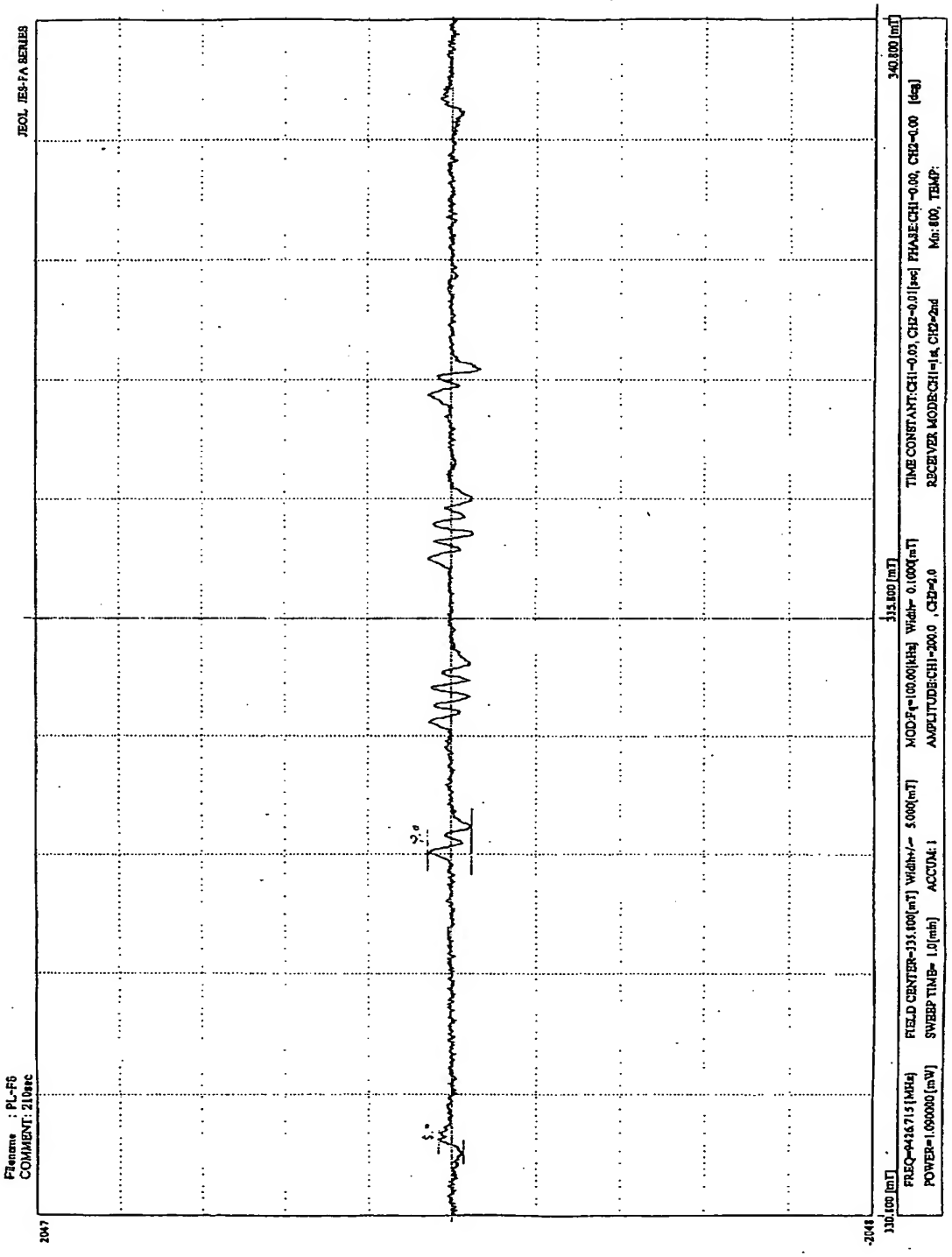
【図１４】


JEOL JES-FA SERIES
Filename : PL-F3
COMMENT: 210sec
2047
-2048
330.800 [mT]
335.800 [mT]
340.800 [mT]
FREQ=9436.782 [MHz] FIELD CENTER=335.800[mT] Width+/-= 5.000[mT] MOD.Fq=100.00[kHz] Width= 0.1000[mT] TIME CONSTANT:CH1=0.03, CH2=0.01[sec] PHASE:CH1=0.00, CH2=0.00 [deg]
POWER=1.000000 [mW] SWEEP TIME= 1.0[min] ACCUM: 1 AMPLITUDE:CH1=2000 , CH2=2.0 RECEIVER MODE:CH1=1st, CH2=2nd Max 800, TEMP:

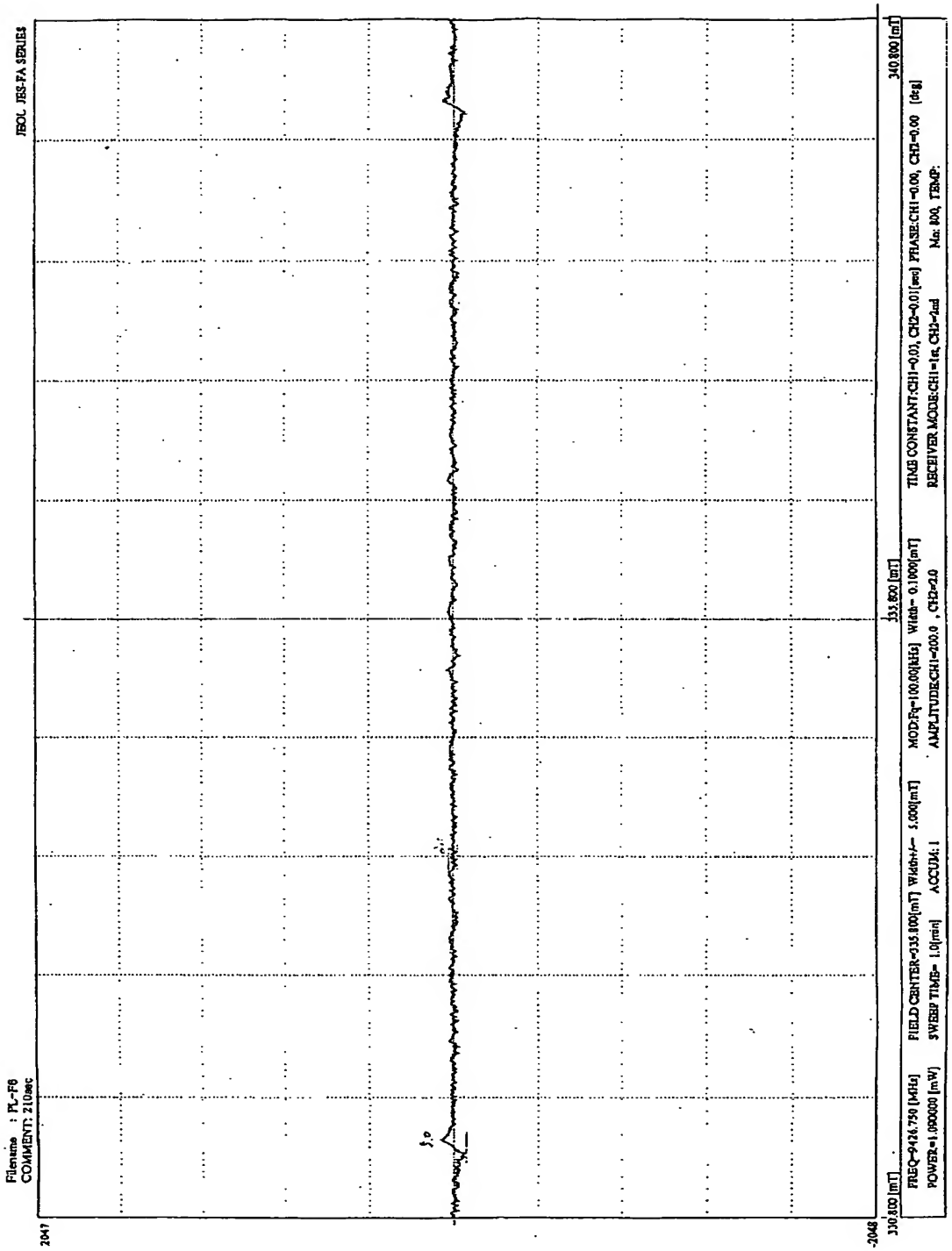
【図１５】


JEOL JES-FA SERIES
Filename : PL-P4
COMMENT: 210sec
2047
-2041
320.000 [mT]
335.800 [mT]
340.800 [mT]
FREQ=9426.453 [MHz]
POWER=1.000000 [mW]
FIELD CENTER=333.100[mT] Width/div= 5.000[mT]
SWEEP TIME= 1.0[min] ACCUM: 1
MOD.Fq=100.00[kHz] Width= 0.1000[mT]
AMPLITUDE:CH1=200.0 , CH2=2.0
TIME CONSTANT:CH1=0.03, CH2=0.01[sec] PHASE:CH1=0.00, CH2=0.00 [deg]
RECEIVER MODE:CH1=1st, CH2=2nd
Mn: 800, TEMP:

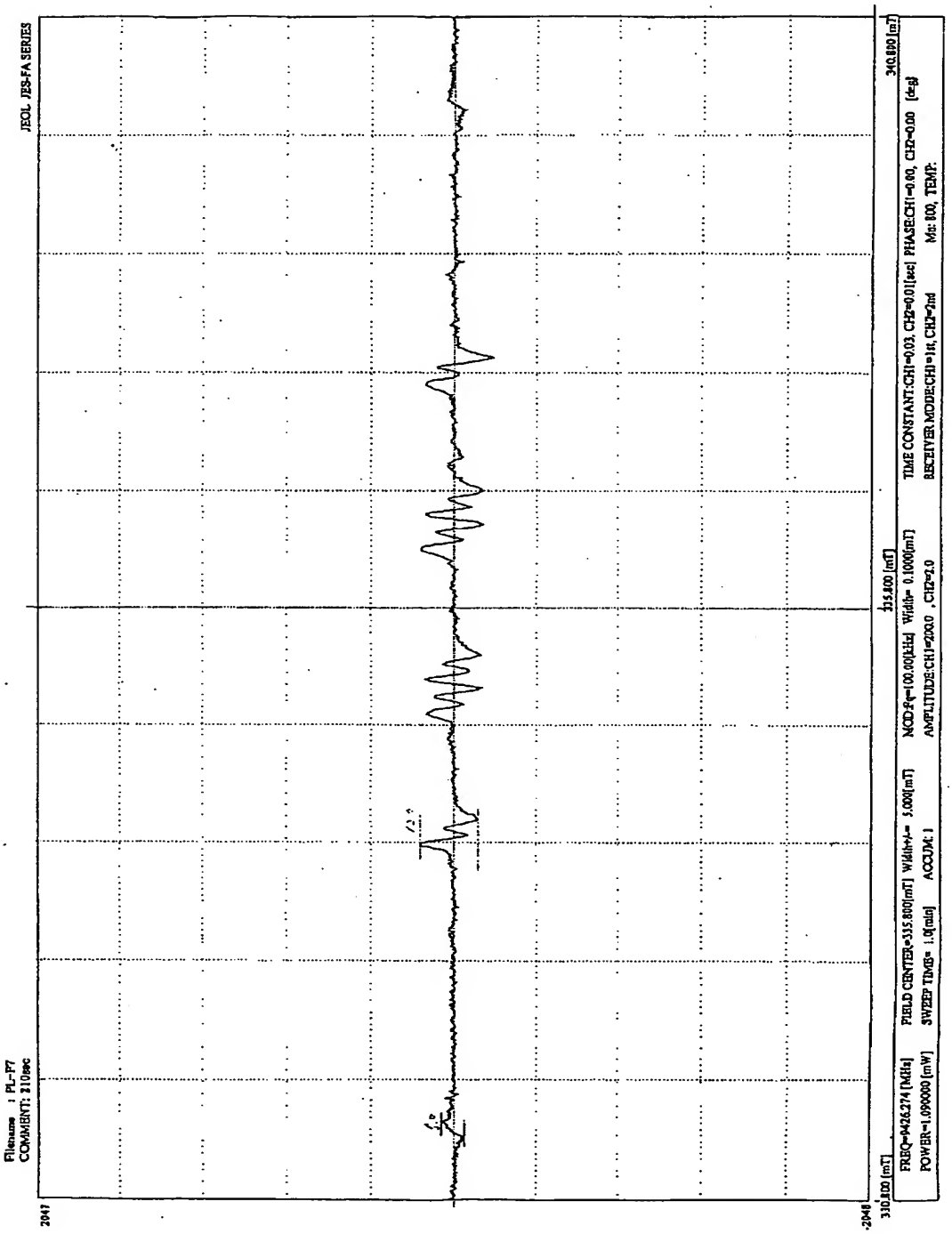
【図１６】


JEOL JES-FA SERIES
Filename : PL-F6
COMMENT: 210sec
2047
-2048
330.100 [mT]
335.100 [mT]
340.100 [mT]
FREQ=9416.715 [MHz]
POWER=1.000000 [mW]
FIELD CENTER=335.100[mT] Width+/- 5.000[mT]
SWEEP TIME= 1.0[min] ACCUM: 1
MOD.Fq=100.00[kHz] Width= 0.1000[mT]
AMPLITUDE:CH1=200.0 ,CH2=2.0
TIME CONSTANT:CH1=0.03, CH2=0.01[sec] PHASE:CH1=0.00, CH2=0.00 [deg]
RECEIVER MODE:CH1=1st, CH2=2nd Mn: 800, TEMP:

【図１７】


JEOL JES-FA SERIES
Filename : PL-F6
COMMENT: 210sec
2047
-2048
330.800 [mT]
335.800 [mT]
340.800 [mT]
FREQ=9426.750 [MHz]
FIELD CENTER=335.800[mT] Width+/- 5.000[mT]
MOD.Fq=100.000[kHz] Width= 0.1000[mT]
TIME CONSTANT:CH1=0.01, CH2=0.01[sec] PHASE:CH1=0.00, CH2=0.00 [deg]
POWER=1.000000 [mW]
SWEEP TIME= 1.0[min] ACCUM.: 1
AMPLITUDE:CH1=200.0 , CH2=2.0
RECEIVER MODE:CH1=1st, CH2=2nd No: 100, TEMP:

【図１８】



フロントページの続き

Ｆターム(参考) 4B050 CC01 DD03 EE04
4B065 AA71X AC14 BA22 BC06
BC08 BC26 BC48 CA28